Laboratory Investigations

Metabolic events with spontaneous malignant hyperthermia crisis in an anaesthetized pig

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Purpose: To analyze metabolic changes associated with a fulminant malignant hyperthermia (MH) crisis developed spontaneously in an MH susceptible pig which was part of 12 pigs undergoing metabolic investigation (six MH susceptible and six controls) and had been anaesthetized with a non-triggering agent (pentobarbitone).

Methods: The pig was placed in a cradle and then inserted into a 4.7 T magnet bore. The semi-membranosus muscle was submitted to three repetitive stimulation-recovery sessions. 31-P magnetic resonance spectra and mechanical data were recorded.

Results: The pig developed a non-rigid MH crisis during recovery from the second set of experiments. Although no mechanical work was performed, dramatic metabolic changes were noted. Twitch tension decreased progressively reaching zero while mouth temperature continuously increased to 44.5°C. Phosphocreatine (PCr) consumption was coupled to Pi accumulation. Also, a marked intracellular acidosis and a large accumulation of phosphomonoesters (PME) were observed, probably as a result of massive glycolysis activation. Interestingly, ATP level remained constant.

Conclusion: These irreversible mechanisms may constitute a metabolic dead-end coupling calcium pumping ATP-consuming processes and ATP synthesis through PCr breakdown and anaerobic glycolysis. They do not differ from metabolic changes previously reported in rigid forms of MH crisis.

Objectif : Analyser les changements métaboliques associés à une crise d'hyperthermie maligne fulminante (HM) spontanée survenue chez un porc sensible faisant partie d'un groupe de 12 porcs soumis à une étude métabolique (six HM sensibles et six contrôles) et anesthésiés avec un agent non déclenchant (le pentobarbitone).

Méthodes : Immobilisé dans un panier, le porc a été inséré dans un champ magnétique de 4,7 T. Son muscle semi-membraneux a subi trois sessions de stimulations-récupérations répétées. Les spectres de résonance magnétique 31-P et les données mécaniques ont été enregistrés.

Résultats : Le porc a développé une crise d'HM non rigide pendant la récupération de la seconde série d'épreuves. Même en absence de travail mécanique, on a pu noter des changements métaboliques remarquables. La tension du twitch a diminué progressivement pour atteindre zéro alors que la température orale augmentait à 44,5°C. La consommation de la phosphocréatinine (PCr) était couplée à l'accumulation de Pi. En outre, on observait une acidose intracellulaire importante et une forte accumulation de phosphomonoesters (PME), résultats probables d'une activation massive de la glycolyse. Il est intéressant de mentionner que le niveau d'ATP est demeuré constant.

Conclusion : Ces mécanismes irréversibles peuvent constituer les processus métaboliques terminaux consommateurs d'ATP du couplage de la pompe calcique et de la synthèse par dégradation de PCr et glycolyse anaérobique. Ils ne sont pas différents des changements métaboliques antérieurement observés pendant les crises d'HM avec rigidité.

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SINCE the first description of the malignant hyperthermia (MH) syndrome in pigs,[1] several reports have addressed the metabolic and physiological conditions associated with MH.[2-7] Various effectors have been used to trigger an MH episode, including pharmacological agents such as succinylcholine, halothane or both, and heat.[2,3] Metabolite concentrations have been measured in blood, on muscle biopsies or using less invasive methods such as 31-phosphorus magnetic resonance spectroscopy (MRS).[8] The physiological signs of MH have been related to variations in body temperature associated with muscle rigidity, hyperventilation, changes in heart rate, PO_2, PCO_2, pH, enzyme and ion blood concentrations.[4,9,10] From a metabolic point of view, phosphocreatine (PCr) breakdown associated with sugar-phosphate accumulation has been reported as a sign of metabolic hyperactivation.[8] However, no data are available on the metabolic and mechanical changes associated with a spontaneous non rigid porcine MH crisis. In the course of a study designed to investigate metabolic and mechanical characteristics of MHS muscles of pigs *in vivo*, using a standardized experimental protocol, one of six MHS pigs under study developed a spontaneous and fatal non rigid MH crisis. Although our results pertain to a single pig, we found it interesting to report the metabolic and mechanical alterations recorded throughout a non rigid, spontaneous porcine MH crisis in order to provide data which, so far, have not been available.

Methods

Animals

This study was approved by the local Animal Investigation Committee. The animal that developed the MH crisis was a piglet from a synthetic line related to Pietrain. He was previously tested as MH sensitive by halothane inhalational challenge (halothane 3%, five-minute test period) and his weight was 28 kg. Fifteen minutes after sedation with 20 $mg \cdot kg^{-1}$ ketamine, anaesthesia was induced with 7.5 $mg \cdot kg^{-1}$ sodium pentobarbitone *iv*. After tracheal intubation, the lungs were ventilated using an automatic spirometer (Pesty-Technomed SA, Aubervilliers-France) and deep anaesthesia was maintained by pentobarbitone boluses iv. The skin of the posterior leg was surgically removed and the *semimembranosus* muscle surface was exposed. A homebuilt double tuned (31-P and 1-H) surface coil was positioned over the muscle. Stimulation electrodes were impaled into the muscle mass. The distal muscle tendon was cut at the bone level and connected to a mechanical transducer with a non extensive halyard. Temperature probes were inserted into the muscle and deeply into the piglet mouth. The piglet was then placed in a non magnetic cradle and inserted into the magnet bore in order to position the surface coil at the magnetic centre. The same experimental device and protocol were used for 12 piglets (six MH sensitive and six controls).

Acquisition of magnetic resonance spectra

Magnetic resonance experiments were conducted in a 30 cm bore 4.7T horizontal magnet equipped with a Biospec spectrometer (Bruker-Biospec 47/30). A 3 cm-diameter homebuilt surface coil was positioned over the muscle mass. The coil was tuned and matched for 1-H (200.14 MHz) and 31-P (80.9 MHz) outside the magnet. Optimization of the field homogeneity was performed on proton free induction decay (FID) of water and fat signals. 31-P FIDs were accumulated, following 100 μsec radiofrequency pulses, in blocks of 30 scans with a repetition time of two seconds. Electrical stimuli were gated in order to be applied during the delay separating sequential MRS acquisitions.

Processing of magnetic resonance spectra

To improve the signal-to-noise ratio, blocks of 30 scans were added in groups of two before Fourier transformation giving a time resolution of two minutes. Signal areas were measured by integration of the respective resonances with the AUTOCAD software (1989 Autodesk AG, version 10.0 IBM PC) after digitization on a Schlumberger-Benson 6453 digitizer (Créteil-France). The perimeter of each signal was defined and fitted with at least 10 data points. Relative concentrations of adenosine triphosphate (ATP), phosphocreatine (PCr), inorganic phosphate (Pi) and phosphomonoesters (PME) were calculated after correction for partial saturation. Saturation factors were calculated comparing the partially saturated spectra recorded using a twenty sec repetition time. Results were expressed as % of PCr surface measured at rest (100%). Intracellular pH was calculated from the chemical shift of the Pi signal relative to PCr at −2.45 ppm with respect to 85% $H_3 PO_4$.[11]

Temperature measurements

Temperature was recorded from two platinum probes (PT 100, Bioblock Scientific, Illkirch, France) placed deep in the mouth and in the muscle. Another probe was positioned in the cradle in order to follow the ambient temperature inside the magnet. All probes were connected to a Macintosh II Apple Computer (Cupertino, USA), through an Analog Device 5B interface I (Issy les Moulineaux, France). Temperature measured deep into the mouth was considered as an index of central temperature.

Mechanical records

Twitch tensions were elicited by electrical stimulation (50 v, 100 mA, 2 msec) of the muscle mass delivered by a stimulator (WP Instruments Inc. model 302, New Haven, USA). Tensions were recorded with a Sedeme AG-20 daN mechano-transducer (Les Ulis, France) interfaced to a Macintosh II computer. Maximal twitch tension was reached by gradual increase of the muscle length and experiments started when the resting muscle tension was stable. Mechanical work was elicited by trains of supramaximal stimulations at 0.1 Hz and 1 Hz successively.

Fatigue test

Once the resting tension was stable, the 40 min fatigue test was started. Supramaximal stimulation was delivered first at a frequency of 0.1 Hz for 10 min, then at a frequency of 1 Hz for 10 min and 20 min recovery was allowed. The test was repeated three times. The duration of the protocol was 135 min while the animal was maintained under deep anaesthesia.

Results

We will mainly describe the chronology of metabolic and mechanical events surrounding the non-rigid MH crisis developed by one of the MHS pigs during the second fatigue test. A typical series of 31-P MR spectra recorded on this pig is presented in Figure 1. The corresponding time-dependent evolutions of phosphorus metabolites and pH are presented in Figures 2 and 3. During the three fatigue tests, the sum of all phosphorylated compounds detected on the spectra remained constant suggesting that metabolic changes were directly linked to muscle stimulation and not secondary to possible changes in spin-lattice relaxation times of the 31-P nuclei borne by the phosphorylated metabolites (Figure 3). The metabolic data recorded on the pig which underwent the MH crisis compared to controls are summarized in Table I.

First fatigue test

At the beginning of the first fatigue test, maximal twitch tension was 4 kg (Figure 4). Intracellular pH was neutral as expected (Table I) and muscle temperature was close to 37°C (Figure 5). During stimulation, although the signal from the muscle temperature probe was altered by electrical currents triggering muscle stimulation, we noted that muscle and mouth temperature varied in the same way based on the measurements performed between the stimulation pulses.

During 0.1 Hz stimulation, PCr level did not vary whereas an intramuscular alkalosis from 6.99 to 7.04 was recorded. The mechanical profile did not change (Figure 4). Larger metabolic changes were recorded throughout the 1 Hz stimulation period.

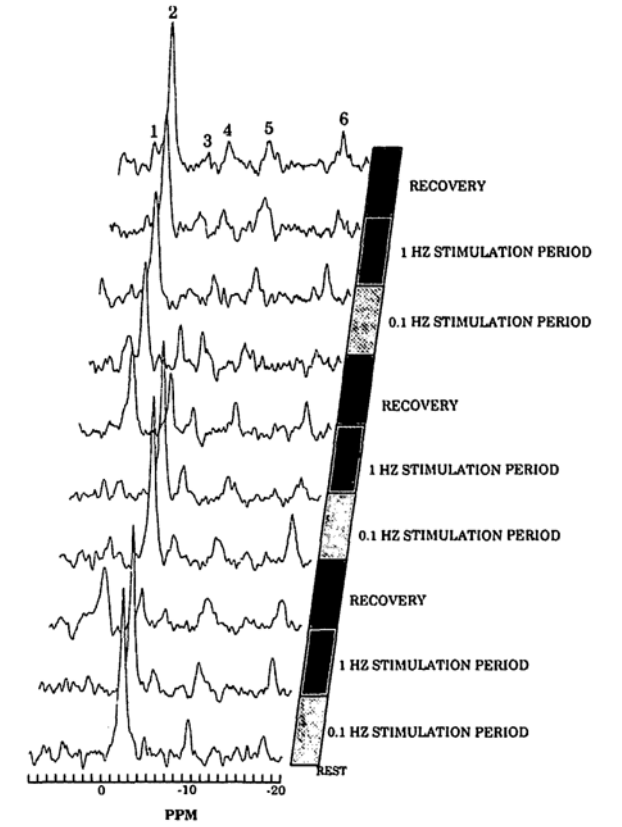

FIGURE 1 Typical 31-P MR spectra recorded throughout the three fatigue tests from the semimembranosus muscle of the pig which underwent a MH crisis. Resonances are assigned as follows: 1: phosphomonoesters (PME), 2: inorganic phosphate (Pi), 3: PCr, 4,5 and 6: δ, α and ß phosphate groups of ATP.

Phosphocreatine consumption was 61% of the initial level and a 0.4 pH unit decrease was recorded (Table I). Twitch tension (Figure 4) was reduced contrary to that observed for control animals.

The accumulation of PME was maximum corresponding to 3.7 times the initial level at the end of the 1 Hz stimulation period. The ATP level remained constant throughout the first fatigue test. Muscle temperature hovered between 37 and 38°C. This first fatigue test did not reveal any differences among all pigs, although a trend towards a larger amplitude of the metabolic variations was found for the pig which subsequently developed the MH crisis. Recovery to the initial levels was observed during the resting period between the two first fatigue tests.

Second fatigue test

The magnitude of PCr and pH changes during the 0.1 Hz and 1 Hz stimulation periods were similar to those recorded throughout the first fatigue test (Table I). During the 1 Hz stimulation period, the muscle tem-

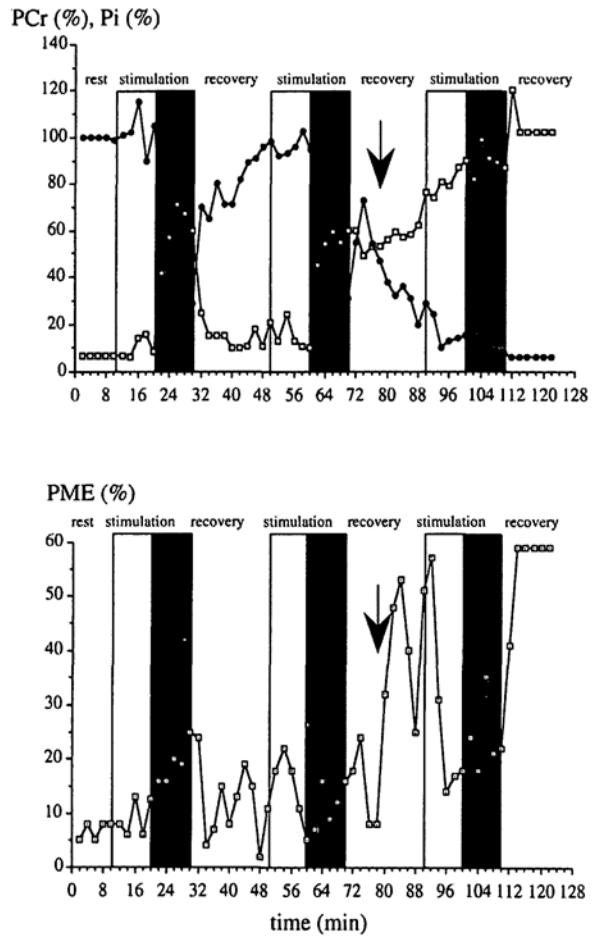

FIGURE 2 Time-dependent evolution of PCr, Pi (top) and PME (bottom) contents throughout the three fatigue tests for the pig which underwent an MH crisis. The arrow indicates triggering of MH crisis. PCr profiles are shown in black circles and open squares designate Pi.

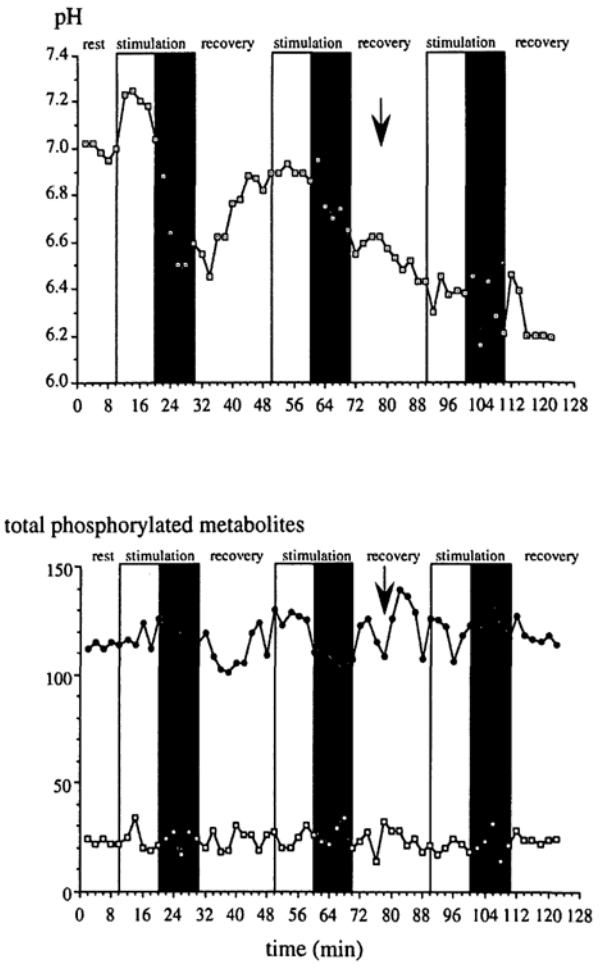

FIGURE 3 Time-dependent evolution of pH (top) and the sum of all phosphorylated metabolites (bottom) i.e., PCr, Pi, ATP and PME (black circles) and the ATP content (white squares) throughout the three fatigue tests. The sum remains constant throughout the experimental protocol. The arrow indicates the triggering of MH crisis.

perature increased abnormally, reaching 38.8°C. This upward trend continued during the following 20 min of rest. Two large temperature jumps were noted throughout the recovery period; at the 9th min of recovery (from 39.5 to 40.6°C) and at the last min of recovery (from 40.9 to 43°C). After an initial recovery of Pi and PCr levels during the first two minutes of the recovery period, an abnormal build-up of Pi coupled to PCr breakdown was recorded whereas control animals recovered towards the resting level. At the same time, a marked intracellular acidosis was observed reaching a pH of 6.45 at the beginning of the third fatigue test. During the second half of the recovery period, a transient contracture corresponding to 25% of the maximal twitch tension was noted (Figure 4).

Third fatigue test

When the last fatigue test was triggered, the animal temperature reached 44.5°C and remained at this level until death. The twitch tension was reduced to 36% of its initial value and was close to zero at the end of the 1 Hz stimulation period. Intracellular pH and PCr continuously decreased. On the contrary to what was observed throughout the first two fatigue tests, a large acidosis (pH = 6.38) was noted during the 0.1 Hz stimulation period. At the end of 1 Hz stimulation, the pH value was 6.21 and PCr level was 6% of the initial level. The PME accumulation was biphasic, first reaching about eight times the initial level after six minutes of stimulation at 0.1 Hz, then, decreased quickly until the beginning of the 1 Hz stimulation period: PME accumulation was maximum at the end of the test (Table I). Surprisingly, ATP concentration remained unchanged. All metabolic variables concurred to pinpoint the 6th minute in the second recovery interval as the starting point of the MH crisis in this susceptible pig.

TABLE I Metabolic parameters recorded throughout the three fatigue tests for the six control pigs and the pig which developed the MH crisis.

		Rest		
	PCr	100 *[100 ± 0]*		
	pH	6.99 *[7.00 ± .01]*		
	ATP	22.8 *[23.6 ± 2.3]*		
	PMEr	6.8 *[5.4 ± 2.5]*		
	mouth temperature	37.5 *[38 ± .2]*		
		end of 0.1 Hz stimulation period	*end of 1 Hz stimulation period*	*end of recovery period*
1st fatigue test	PCr	105 *[80 ± 10]*	29 *[28 ± 8]*	98 *[97 ± 6]*
	pH	7.04 *[7.00 ± 0.10]*	6.60 *[6.67 ± 0.12]*	6.90 *[6.96 ± 0.05]*
	ATP	92 *[96 ± 5]*	105 *[99 ± 6]*	108 *[102 ± 6]*
	PME/PMEr	1.9 *[1.5 ± 0.5]*	3.7 *[3 ± 1]*	1.6 *[1.2 ± 0.5]*
	mouth temperature	37.1 *[38.5 ± .1]*	37.9 *[38.2 ± .5]*	37.7 *[38.2 ± .3]*
2nd fatigue test	PCr	95 *[70 ± 7]*	31 *[24 ± 13]*	29 *[87 ± 6]*
	pH	6.85 *[6.98 ± 0.05]*	6.65 *[6.85 ± 0.1]*	6.45 *[7.1 ± 0.1]*
	ATP	104 *[97 ± 7]*	88 *[89 ± 10]*	92 *[95 ± 8]*
	PME/PMEr	0.7 *[1.2 ± 0.3]*	2.4 *[1.9 ± 0.2]*	7.5 *[2.3 ± 0.05]*
	mouth temperature (°C)	37.9 *[37.9 ± .5]*	38.8 *[38.5 ± .4]*	43.4 *[38.1 ± .3]*
3rd fatigue test	PCr	15 *[70 ± 5]*	8 *[37 ± 6]*	6 *[85 ± 9]*
	pH	6.38 *[7.00 ± 0.09]*	6.20 *[6.85 ± 0.10]*	6.21 *[6.98 ± 0.10]*
	ATP	79 *[83 ± 6]*	92 *[90 ± 4]*	105 *[97 ± 3]*
	PME/PMEr	2.6 *[1.8 ± 0.3]*	3.3 *[2.2 ± 0.4]*	8.7 *[2.5 ± 0.3]*
	mouth temperature (°C)	44.3 *[38.5 ± .4]*	44.5 *[38.1 ± .1]*	44.5 *[38.5 ± .5]*

Control values are indicated in brackets as mean ±_ SEM (n = 6). At rest, ATP and PMEr are expressed as percent of the PCr content measured at rest. Throughout the three fatigue tests, PCr and ATP values are expressed as percent of the respective values recorded at rest. PME/PMEr indicated the amount of PME measured as compared to the resting PME level.

Discussion

Direct analysis of the sequence of events surrounding this MH crisis developed by the MHS pig while in the magnet of the MR spectrometer is of particular interest for several reasons. First, this crisis was spontaneous i.e., not triggered by a pharmacological agent such as halothane or succinylcholine. Moreover, it was non rigid, as sometimes reported in humans. The analysis of such a crisis could provide information about the pathogenesis of MH episodes and the identity of several possible triggering factors.

The first detectable signs of the spontaneous and non rigid MH crisis were the lack of reversibility in metabolic process, i.e., PCr breakdown, and intracellular acidosis coupled to PME accumulation at the end of the second fatigue test. These signs were preceded by a trend towards lower pH values during the second fatigue test which could correspond to early metabolic changes announcing the MH crisis. It is noteworthy that energy consumption and metabolic activation were recorded whereas no mechanical work was produced as a sign of net ATP-consuming processes. After six minutes

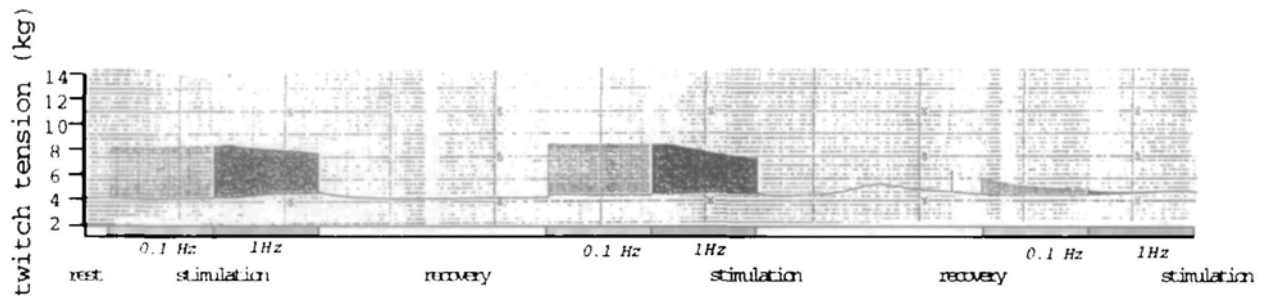

FIGURE 4 Time-dependent evolution of the twitch tension of the semimembranosus muscle submitted to the three fatigue tests. A slight and evanescent contracture appeared during the second recovery period.

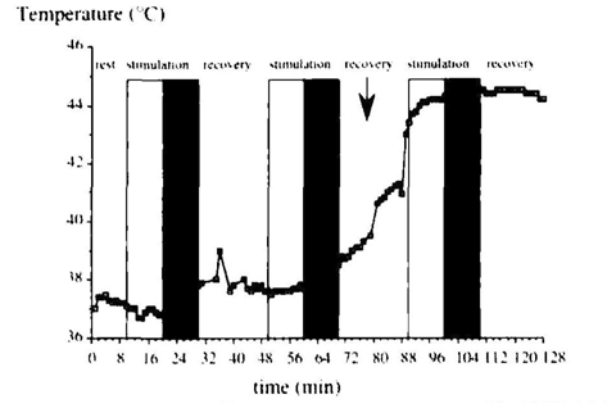

FIGURE 5 Time-dependent evolution of mouth temperature throughout the three fatigue tests. The temperature was recorded from a probe inserted deeply within the mouth of the pig and can be consequently considered as an index of the central temperature. The arrow indicates the triggering of MH crisis.

of rest, pH remained low at 6.6 and both PCr and Pi concentrations did not follow the normal recovery profile. At this time, the PME signal emerged on the MR spectrum and the increase in temperature was continuous but modest (0.5 to 0.8°C). The MR profile already revealed signs of hypermetabolism with an activation of glycolytic processes. The crisis developed during the 20 min of rest and reached its maximum at the onset of the third fatigue test when electrical stimulation reduced the intracellular pH to 6.2, completed the depletion of PCr and the overshoot of Pi while increasing the animal temperature to 44.5°C. The intracellular acidosis coupled to PME accumulation indicates massive glycolytic stimulation in agreement with previous results *in vitro*[12] and with blood lactate production known as an early indicator of porcine MH crisis.[4] Excess glycogenolysis leads to accumulation of PME, due mainly to accumulation of glucose 6-phosphate and fructose 6-phosphate as a result of an imbalance between glycogen phosphorylase and phosphofructokinase (PFK) activities.[13] This activation could be mediated by the large amount of calcium released during MH. Indeed, halothane-induced MH crisis is associated with an enhanced rate of calcium release due to the interaction between halothane and the calcium release channel. This association stimulates metabolic processes such as ATP-consuming calcium pumping and it has been shown that myoplasmic calcium changes were the first detectable signs of a porcine malignant hyperthermia episode preceding metabolic and clinical symptoms.[14,15] Although the MH crisis we describe was spontaneous, it is likely that the irreversible processes of PCr breakdown and intracellular acidosis reflect this futile ATP-consuming calcium pumping cycle. Also, Pi may act as a substrate of glycogen phosphorylase for glycogen utilization, accounting for the massive glycolytic stimulation. In addition, PME accumulation can be associated with uncontrolled cycling of fructose 6-phosphate through PFK and fructose diphosphatase thereby leading to heat production. At the end of the protocol, PCr amounted to 6% of the initial level and PME content reached about nine times the resting level in agreement with values previously recorded on cryoprobe biopsies after 30 min MH.[6] Surprisingly, and contrary to reports in man during exhaustive exercise,[16] ATP content remained constant throughout the protocol. This may explain the failure to observe any contracture during the crisis considering that ATP is necessary for the decomplexation of cross-bridges. The relationship between net ATP consumption and muscle rigidity remains obscure. Decanniere *et al.* reported that four of 17 pigs displayed muscle rigidity after halothane-induced porcine MH crisis whereas the minimum ATP level was 80% of the resting concentration.[8] Also, continuous exposure of an MHS pig to halothane resulted in dramatic PCr consumption, down to 10% of the initial level, associated with complete rigidity and death.[17] Finally, according to

Ryan *et al.*, rigidity occurs when intracellular calcium concentration is high (>1 mM) and is no more regulated by calcium ATPases due to low level of ATP.[15]

Another interesting aspect of this report is the lack of rigidity which accompanied the MH crisis in the sensitive pig. Non-rigid forms of MH crisis are known in humans[18] and pigs.[19,20] In our study, the same animal displayed two different MH reactions. During the halothane inhalational test, the animal was classified as MHS as limb rigidity with contracture was observed. On the contrary, under anaesthesia, no rigidity was associated with the MH crisis. Although no clear explanation can be provided at the present time, it is interesting to note that, at least in pigs, susceptibility to anaesthestics varies widely. Gronert *et al.* have emphasized the difficulty of triggering an MH crisis by sevoflurane, a halogenated agent, in the presence of thiopentone.[10] In fact, succinylcholine is necessary to trigger a crisis. Also, ryanodine injection in pigs triggered a typical MH crisis accompanied by muscle rigidity whereas, in the same animal, MH crisis developed without muscle rigidity if the pig was previously anaesthetized with thiopentone.[21] Under our experimental conditions, the pig developed an MH crisis when anaesthesia had been induced and maintained by sodium pentobarbitone which is considered a safe anaesthetic.

During this spontaneous MH crisis, the temperature increased markedly, just after the first abnormal metabolic signs, reaching 43°C at the beginning of the third fatigue test. Thereafter, the increase was less pronounced despite continuous metabolic alterations. It has been previously demonstrated that the primary site of heat production in porcine malignant hyperthermia is skeletal muscle.[22] More recently, it has been reported that vital organs such as the liver, rather than skeletal muscle, were the primary sites of heat production occurring mainly during the initial stage of porcine MH.[23] On the basis of comparative analysis of oxygen consumption and temperature changes during porcine MH, it has been shown that anaerobic metabolism is the major source of heat production.[24] Under our experimental conditions, taking into account that no triggering agents were used, heat production could have been induced either by electrical stimulation or by the abrupt production of catecholamines. We had positioned the stimulation electrodes within the muscle mass and used low stimulation frequency in order to prevent any systemic hormonal reaction from occurring. However, the direct electrical stimulation of the muscle could stimulate motor and sympathetic nerve endings thereby increasing local blood epinephrine concentrations and triggering porcine MH via anaerobic glycolysis activation. Based on the observed increase in circulating plasma norepinephrine concentrations during an MH episode, several investigators have claimed that catecholamines are primarily involved in initiating the MH crisis or at least help in providing the proper physiological setting for MH to occur.[25,26] On the other hand, it has been shown that hypermetabolic changes precede signs of increased sympathetic activity suggesting that norepinephrine does not initiate nor mediate the onset of MH crisis.[9,27,28] Epinephrine infusion of rabbit exercising muscle has been shown to induce metabolic alterations which are very similar to those reported here[29] suggesting that in our observation, heat production could have been mediated by local epinephrine increase.

Finally, we will address the issue of increased ambient temperature as a possible triggering factor since the heat produced by the pig could not be easily dissipated in the cradle where the basal temperature was 30°C. It has been reported that rectal temperature up to 41°C or ambient temperature of 45°C can trigger porcine MH.[2,3] Under our experimental conditions, the animal temperature was only 39°C when the first metabolic sign of MH was detected but ambient temperature (inside the magnet bore) was slightly elevated (32°C). It could be hypothesized that metabolic hyperactivation is mediated by an epinephrine bolus elicited by environmental factors (temperature).

In conclusion, the increase in ambient temperature was likely to be the first triggering factor of the crisis. The ensuing hypermetabolic activation can be assigned to a futile-energy consuming cycle aiming at regulating increase influx of calcium. Epinephrine production was probably an additional activating factor of this futile cycle. It is noteworthy that the metabolic events reported in this study throughout a non-rigid MH episode are comparable to that previously reported during a rigid MH crisis in pigs.[8] Considering that the genetic defect is unique in pigs and that rigid and non-rigid forms have been described, it could be postulated that rigid and non-rigid forms of MH in humans (associated or not with the RYR 1 gene) could also be the consequence of the same genetic defect. It is well known that development and intensity of MH crises in humans and pigs depend on the pharmacological agents used for anaesthesia. Combination of succinylcholine and halothane triggered a fatal rigid MH crisis in a member of a MHS family whereas, for his brother, a MH non rigid crisis was recorded when anaesthesia was solely induced by halothane (personal communication from Dr Laxenaire-Nancy).

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